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Outbreak of Highly Pathogenic Avian Influenza in Japan and Anti-Influenza Virus Activity of Povidone-Iodine Products

Hiroshi Ito[a] Toshihiro Ito[a] Muneo Hikida[c] Junko Yashiro[c] Akira Otsuka[c]
Hiroshi Kida[d] Koichi Otsuki[b]

Departments of [a]Veterinary Public Health and [b]Veterinary Microbiology, Faculty of Agriculture, Tottori University, Tottori, [c]Meiji Seika Co. Ltd., Tokyo, and [d]Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan

Key Words
Povidone-iodine · Highly pathogenic avian influenza · Virucidal activity · Antiseptics

Abstract
Objectives: On January 12, 2004, an outbreak of highly pathogenic avian influenza, caused by the H5N1 strain, occurred in a one-layer flock in Yamaguchi Prefecture, Japan. It had been 79 years since the last outbreak of avian influenza was confirmed in Japan. By February, 3 additional outbreaks had occurred (1 in Oita Prefecture and 2 in Kyoto Prefecture). Influenza viruses are enveloped viruses and are relatively sensitive to inactivation by lipid solvents, such as detergents. Infectivity is also rapidly destroyed by ether, sodium hypochlorite, povidone-iodine (PVP-I), peracetic acid and alcohol. However, these antiviral effects were only tested against human influenza A viruses. In the present study, the antiviral activity of PVP-I products against H5, H7 and H9 avian influenza A viruses, which had recently been transmitted to humans, were investigated. ***Methods:*** The in vitro antiviral activity of PVP-I products (2% PVP-I solution, 0.5% PVP-I scrub, 0.25% PVP-I palm, 0.23% PVP-I gargle, 0.23% PVP-I throat spray and 2% PVP-I solution for animals) against avian influenza A viruses [a highly pathogenic avian influenza virus, A/crow/Kyoto/T2/04 (H5N1; $10^{6.5}$ EID_{50}/0.1 ml), and 3 low pathogenic avian influenza A viruses, A/whistling swan/Shimane/499/838 (H5N3; $10^{4.8}$ EID_{50}/0.1 ml), A/whistling swan/Shimane/42/80 (H7N7; $10^{5.5}$ EID_{50}/0.1 ml) and A/duck/Hokkaido/26/99 (H9N2; $10^{4.8}$ EID_{50}/0.1 ml)] were investigated using embryonated hen's eggs. ***Results/Discussion:*** Viral infectious titers were reduced to levels below the detection limits by incubation for only 10 s with the PVP-I products used in this study. These results indicate that PVP-I products have virucidal activity against avian influenza A viruses. Therefore, the PVP-I products are useful in the prevention and control of human infection by avian influenza A viruses.

Introduction

In January 2004, an outbreak of highly pathogenic avian influenza occurred in a one-layer flock in Yamaguchi Prefecture, Japan. It had been 79 years since the last outbreak of avian influenza was confirmed in Japan. By February, 3 additional outbreaks had occurred, 1 in Oita Prefecture and 2 in Kyoto Prefecture.

Highly pathogenic avian influenza is an acute infectious disease that causes systemic symptoms in poultry [1]. Although the symptoms are varied, the mortality rate

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Toshihiro Ito
Department of Veterinary Public Health, Faculty of Agriculture
Tottori University, 4-101 Koyama-cho Minami
Tottori 680-8553 (Japan)
Tel./Fax +81 857 31 5437, E-Mail toshiito@muses.tottori-u.ac.jp

is high and the disease is highly contagious. Therefore, when it occurs, the poultry industry suffers devastating damage.

The virus contains two surface glycoprotein spikes, hemagglutinin and neuraminidase. Influenza A viruses are classified serologically based on their hemagglutinin and neuraminidase subtype combinations.

It was thought that avian influenza virus does not transmit directly from birds to humans. However, in 1997, highly pathogenic H5N1 avian influenza virus was transmitted from chickens to humans in Hong Kong [2, 3]. Thus far, 18 cases have been identified and of those, 6 died. In addition, that low pathogenic H9N2 avian influenza virus infected 2 people in Hong Kong was reported in 1999 [4]. During 2003, an avian influenza outbreak caused by the highly pathogenic H7N7 virus occurred in the Netherlands and human infection with this virus was also reported [5].

Therefore, avian influenza is now thought to be an important zoonosis, not a simple avian disease [6–8].

Summary of the Outbreaks of Highly Pathogenic Avian Influenza in Japan

The First Case

In an egg production farm in Ato-Cho, Yamaguchi Prefecture, Japan, on December 28, 2003, dead chickens were confirmed in the poultry house No. 1. Since the number of chicken deaths subsequently increased and spread to other houses, the egg farm requested the local Livestock Hygiene Service Center to make a disease diagnosis. On January 11, Yamaguchi Prefecture sent test samples to the National Institute of Animal Health, confirming infection by subtype H5 avian influenza virus. Thus, the chickens were found to be suffering from highly pathogenic avian influenza infection.

After confirmation of the outbreak, Yamaguchi Prefecture devised the necessary epidemic prevention measures including the culling of all chickens at the farm, disinfection, restrictions on movement at other farms in the vicinity and implementation of epidemiological surveys.

The movement of chickens and other domestic fowl as well as objects that could potentially cause the spread of the virus was prohibited within a zone with a radius of 30 km centered around the infected farm.

In virus-free confirmation tests conducted by Yamaguchi Prefecture on February 14, no other infections were confirmed in any of the flocks.

The Second Case

On February 14, 2004, three bantams died at the home of a fancier of pet bantams in Kokonoe-machi, Oita Prefecture of Kyushu Island. Therefore, the breeder reported the event to the local Livestock Hygiene Service Center.

Oita Prefecture sent test samples to the National Institute of Animal Health, and the chickens were confirmed to be infected with the same highly pathogenic avian influenza virus.

As in the case of Yamaguchi Prefecture, as initial epidemic prevention measure, entry into the infected location by outsiders was restricted and the poultry house was disinfected, etc. At the same time, a zone with a radius of 30 km was designated centered around the infected location.

The Third Case

There was an increase in dead chickens in the poultry house No. 8 of an egg production facility in Tanba-cho, Kyoto Prefecture, on February 17, 2004. The infection spread to virtually all of the houses within a few days.

The breeder did not report this to the Livestock Hygiene Service Center while this was occurring. The Service Center, which had received an anonymous telephone call reporting large-scale deaths at the farm, conducted an on-site inspection before dawn on February 27. The virus was also confirmed to be H5.

Kyoto Prefecture also devised the necessary epidemic prevention measures, such as restricted entry to the farm by outsiders, constraints on egg shipments, the culling of the chickens at the farm, disinfection of poultry houses, and restrictions on movement at other farms in the vicinity.

Slaked lime was used to disinfect poultry houses.

However, it became clear meanwhile that infected poultry had already been shipped to chicken processing plants in Hyogo and Aichi Prefectures.

H5 avian influenza viruses were also isolated from a total of 9 jungle crows between May 7 and April 9 in the restricted movement zone of the third outbreak. No other infection of wild birds was confirmed.

The Fourth Case

Since there was a rapid increase in dead chickens on March 3 at a broiler farm in Kyoto Prefecture located about 4 km northeast of the infected farm of the third outbreak, the farm manager notified the Livestock Hygiene Service Center. Kyoto Prefecture sent samples of the dead chickens to the National Institute of Animal Health on March 5 and a disease diagnostic investigation

Table 1. Virucidal activity of PVP-I against influenza viruses

PVP-I product	PVP-I concentration %	Mean titer of remaining viruses (EID_{50}/0.1 ml)			
		HPAI	LPAI		
		H5 (T2)	H5 (499)	H7 (42)	H9 (26)
PVP-I solution	2	–	–	–	–
PVP-I scrub	0.5	–	–	–	–
PVP-I palm	0.25	–	–	–	–
PVP-I gargle	0.23	–	–	–	–
PVP-I throat spray	0.23	–	–	–	–
PVP-I solution for animals	2	–	–	–	–
Control (PBS)	0	$10^{6.5}$	$10^{4.8}$	$10^{5.5}$	$10^{4.8}$

Concentrations indicate the final concentration of each PVP-I product. HPAI = Highly pathogenic avian influenza; LPAI = low pathogenic avian influenza; – = below the detection limit.

was conducted. The result showed that the isolated virus was the same H5 highly pathogenic avian influenza virus.

Since this outbreak was related to the third case in the restricted movement zone, a response was implemented concomitant with the third outbreak without setting a new restricted movement zone.

Thus, a total of 4 poultry-raising facilities had to sacrifice about 275,000 domestic fowl.

Although it was fortunately possible to keep the damage to a minimum in Japan, large-scale outbreaks of the disease were also confirmed in Southeast Asia, and in some countries, for example Thailand and Vietnam, human infections increased. Therefore, those outbreaks not only involved livestock hygiene, but also became an issue of nationwide interest as a public health problem.

Study on Antiviral Activity of Povidone-Iodine Products against Avian Influenza Viruses

Introduction

Influenza viruses are enveloped viruses and are relatively sensitive to inactivation by lipid solvents, such as detergents. Infectivity is also rapidly destroyed by ether, sodium hypochlorite, povidone-iodine (PVP-I), peracetic acid and alcohol. Wutzler et al. [9] demonstrated that PVP-I products exhibited an antiviral effect against human influenza viruses. However, no avian influenza viruses have been tested.

Therefore, in the present study, the antiviral activity of povidone-iodine products against avian influenza viruses was investigated.

Materials and Method

A total of 3 low pathogenic avian influenza strains, A/whistling swan/Shimane/499/83 (H5N3), A/whistling swan/Shimane/42/80 (H7N7) and A/duck/Hokkaido/26/99 (H9N2) viruses, were used. Furthermore, a highly pathogenic avian strain, A/crow/Kyoto/T2/04 (H5N1) which was isolated from a crow during the Kyoto outbreak in 2004, was also used.

These viruses were grown in 10-day-old embryonated chicken eggs at 35°C for 2 days.

The following 6 kinds of PVP-I products were used in this study: 2% PVP-I solution, 0.5% PVP-I scrub, 0.25% PVP-I palm, 0.23% PVP-I gargle, 0.23% PVP-I throat spray and 2% PVP-I solution for animals. Each PVP-I product was diluted with sterilized distilled water just before the examination. The concentration of each PVP-I product which was used for this study was set mainly as that prescribed for usage.

The examination was performed as described by Kawana et al. [10], namely 0.25 ml of each PVP-I product was mixed with 0.25 ml of tested virus and incubated at 25°C for 10 s. The reaction was stopped promptly by adding phosphate-buffered saline containing 0.5% sodium thiosulfate.

Serial 10-fold dilutions of each reaction fluid were inoculated into allantoic cavities of 10-day-old embryonated eggs and incubated for 2 days at 35°C. Virus titers were determined by the hemagglutination test and the method of Reed and Muench [11].

The examination of the highly pathogenic strain A/crow/ Kyoto/T2/04 was carried out in BL3 containments in Hokkaido University.

Results

As shown in table 1, viral infectious titers were reduced to levels below the detection limits by incubation for only 10 s with all PVP-I products used in this study.

Conclusion

These results indicate that PVP-I products have virucidal activity against avian influenza A viruses. Therefore, the PVP-I products are probably useful in the prevention and control of human infection by avian influenza A viruses.

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